UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of Acute Mammalian Toxicity Data in Support of Registration of Rhizopon AA #3 (0.8% a.i.) by HORTUS USA Corporation (ID# 063310-ER; DP Barcode D213970; Submission No. S481627; Chemical Code Number 046701)

TO: Rita Kumar
Regulatory Action Leader
Biopesticides and Pollution Prevention Division (7501W)

FROM: Sheryl K. Reilly, Ph.D., Biologist
Biopesticides and Pollution Prevention Division (7501W)

ACTION REQUESTED: Review of six acute mammalian toxicity data in support of the registration of Rhizopon AA #3 (0.8% a.i.).

CONCLUSIONS: The studies are summarized as follows:

MRID NO.: 435274-05 Acute Oral Toxicity – Rat (152-10)
No signs of toxicity or treatment-related effects were observed during a 2-week observation period in rats given an acute oral limit (5000 mg/kg) dose of Rhizopon AA #3 (0.8%). The **oral LD_{50}** of Rhizopon AA #3 (0.8%) in rats is **> 5000 mg/kg**. The study is **Acceptable** and places the test material in **Toxicity Category IV**.

MRID NO.: 435274-06 Acute Dermal Toxicity - Rabbit (152-11)
An acute limit dose (2000 mg/kg) of Rhizopon AA #3 (0.8%) was applied to the shaved skin of New Zealand white rabbits for 24 hours. Clinical signs included diarrhea and yellow nasal discharge during the 14-day observation period. Erythema at the test site was observed in 2 rabbits on day 1, but cleared by days 7 and 14. None of the rabbits died during the study. The **dermal LD_{50}** of Rhizopon AA #3 (0.8%) in rabbits is **> 2000 mg/kg**. The study is **Acceptable** and places the test material in **Toxicity Category III**.

MRID NO.: 435274-07 Primary Dermal Irritation – Rabbit (152-14)
Rhizopon AA No. 3 (0.8%) was applied (0.5 g moistened with distilled water) to the shaved dorsal trunk area of 6 New Zealand white rabbits. Four hours later, the dressings were removed and the site of application was examined at 30 to 60 minutes and 24, 48, and 72 hours later. Erythema and edema were absent at all observations. Under the conditions of the study, Rhizopon AA No. 3 (0.8%) is **not a skin irritant** in rabbits. The study is **Acceptable** and places the test compound in **Toxicity Category IV.**

MRID NO.: 435274-08 Primary Eye Irritation – Rabbit (152-13)
Rhizopon AA No. 3 (0.8/%) was tested (0.1 ml equivalent) in the eyes of New Zealand white rabbits. The untreated eye of all rabbits served as control. No corneal opacity was observed, but iritis was noted in 2/6 treated eyes at 1 hour; this and cleared by day 1. Conjunctival irritation was observed in 5/6 treated eyes and cleared by day 2. No clinical signs of toxicity were observed. Rhizopon AA No. 3 (0.8%) is **mildly irritating to the eye** of rabbits. The study is classified as **Acceptable** and places the test material in **Toxicity Category III.**

MRID NO.: 435274-09 Acute Inhalation Toxicity – Rat (152-12)
Wistar albino rats were exposed to a gravimetric atmospheric concentration of 2.4 mg/L Rhizopon AA No. 3 (0.8%) for 4 hours. No clinical signs were observed and no animals died during the subsequent 14-day observation period. The **LC_{50}** of Rhizopon AA No. 3 (0.8%) is **> 2.4 mg/L**. The study is **Acceptable** and places the test material in **Toxicity Category IV.**

MRID NO.: 435274-10 Dermal Sensitization, Buehler Method – Guinea Pig (152-15)
Male Hartley guinea pigs were induced once per week (6 hour/treatment) for 3 weeks with either 0.4 g Rhizopon AA No. 3 (0.8%) moistened with 0.1 ml distilled water or 0.2% 1-chloro-2,4-dinitrobenzene (DNCB) as a positive control. Two weeks following the third induction, the test animals were challenged with the moistened test material and the positive controls with a 0.1% concentration of DNCB. Under the conditions of the study, Rhizopon AA No. 3 (0.8%) was **not a contact sensitizer.** The study is **Acceptable.**

The Data Evaluation Reports are attached.

[RHIZOPON AA No. 3 (0.8)] Acute Oral Study (81-1)

EPA Primary Reviewer: Sheryl K. Reilly, Ph.D. _____, Date 6/22/95
EPA Secondary Reviewer: Roy D. Sjoblad, Ph.D. _____, Date 6/27/95
Biopesticides and Pollution Prevention Division (7501W)

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity - Rat (152-10)

TOX. CHEM. NO.: 046701

DP BARCODE: D213970

MRID NO.: 435274-05

TEST MATERIAL: Rhizopon AA No. 3 (0.8% a.i.)

SYNONYMS: None known

STUDY NUMBER: MB 94-4093 A

SPONSOR: HORTUS USA CORP.

TESTING FACILITY: MB Research Laboratories, Inc., P.O. Box 178, Steinsburg and Wentz Roads, Spinnerstown, PA 18968

TITLE OF REPORT: Single Dose Oral Toxicity in Rats

AUTHOR: Daniel R. Cerven

REPORT ISSUED: January 17, 1995 (Study completion date)

EXECUTIVE SUMMARY: Wistar albino rats (5/sex) were given a single 5000 mg/kg gavage dose of Rhizopon AA #3 (0.8% a.i.). No clinical signs of toxicity were observed; no effects on body weight were noted, and no animals died during the 14-day observation period. No apparent treatment-related effects were noted at necropsy. Based on the study results, the **oral LD_{50}** of Rhizopon AA #3 (0.8% a.i.) is **> 5000 mg/kg** (Limit Test). This places the test material in **Toxicity Category IV**.

The study is classified as **Acceptable** and **meets** the requirement of Guideline Number 152-10 (81-1) for an acute oral toxicity study in rats.

A. MATERIALS

1. Test material: Rhizopon AA #3 (0.8% a.i.)

Description: gray powder
Lot/Batch No: unknown
Composition: unknown
Stability: unknown
pH: unknown
Solubility: water

2. Test animals

Species: rat
Strain: Wistar
Age: approx. 3 months
Weight: pretest - 268-300 g (males), 214-231 g (females)
Source: Ace Animals

3. Animal care

Housing: 5/sex/cage in suspended wire mesh cages
Food: Purina Rat Chow #5012, *ad libitum* except for 16-20 hr prior to dosing
Water: *ad libitum*
Acclimation period: at least 7 days
Temperature: "temperature controlled"
Humidity: not reported
Air changes: not reported
Photoperiod: 12 hour light/dark

B. METHODS

After a 16-20 hour fast, Wistar albino rats (5/sex) received a single dose of 5000 mg/kg test material by gavage. The material was mixed with distilled water, and the dose was based on the dry weight of the test material. The body weight of all animals included in the study was recorded immediately prior to dosing, and on days 7 and 14 post-treatment. In addition, the animals were observed for clinical signs of toxicity 1, 2, and 4 hours post-treatment and once daily thereafter for the remainder of the 14 day study. At study end, the animals were euthanized (method not specified) and examined for gross pathology. A control group was not included in the study.

C. RESULTS

1. Mortality

All rats survived through the 14-day observation period of the study.

2. Clinical observations

No clinical signs of toxicity were observed during the study.

3. Body weight

No apparent effects on body weight were observed.

4. Necropsy

All tissues and organs appeared normal at necropsy.

5. LD_{50}

Based on the study results, the rat oral LD_{50} for Rhizopon AA #3 (0.8% a.i.) is > 5000 mg/kg (Limit Test). This places the test material in Toxicity Category IV.

D. Signed Quality Assurance and Good Laboratory Practice statements were present.

EPA Primary Reviewer: Sheryl Reilly, Ph.D. _____, Date 6/22/95
EPA Secondary Reviewer: Roy D. Sjoblad, Ph.D. _____, Date 6/27/95
Biopesticides and Pollution Prevention Division

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity - Rabbit (152-11)

TOX. CHEM. NO.: 046701

DP BARCODE: D213970

MRID NO.: 435274-06

TEST MATERIAL: Rhizopon AA No. 3 (0.8% a.i.)

SYNONYMS: None known

STUDY NUMBER: MB 94-4093 B

SPONSOR: HORTUS USA CORP.

TESTING FACILITY: MB Research Laboratories, Inc., P.O. Box 178, Steinsburg and Wentz Roads, Spinnerstown, PA 18968

TITLE OF REPORT: Acute Dermal Toxicity/LD_{50} in Rabbits

AUTHOR: Daniel R. Cerven

REPORT ISSUED: January 17, 1995 (Study completion date)

EXECUTIVE SUMMARY: In an acute dermal toxicity study, 2000 mg/kg Rhizopon AA #3 (0.8% a.i.) was applied to the shaved skin of five male and five female New Zealand white rabbits for 24 hours and the rabbits observed for 14 days. Diarrhea, yellow nasal discharge, and few feces were observed during the observation period. No significant body weight changes were observed. Erythema, observed in two females on day 1, was absent on days 7 and 14. None of the rabbits died during the study. Based on the study results, the **dermal LD_{50}** of Rhizopon AA #3 (0.8% a.i.) for rabbits is **> 2000 mg/kg**.

The study is **Acceptable** and **meets** the requirements of Guideline Number 152-11 (81-2). The test material is classified in **Toxicity Category III**.

A. MATERIALS

1. Test material: Rhizopon AA No. 3 (0.8% a.i.)

Description: gray powder
Lot/Batch No.: unknown
Composition: unknown
Stability: unknown
pH: unknown
Solubility: water

2. Test animals

Species: rabbit
Strain: New Zealand white
Age: approx. 3 months
Weight: pretest - 2.3-2.5 kg (males), 2.1-2.4 kg (females)
Source: Ace Animals, Boyertown, PA

3. Animal care

Housing: individually in suspended wire cages
Food: Purina Rabbit Chow (Diet No. 5321), assumed *ad libitum*
Water: *ad libitum*
Acclimation period: at least 7 days
Temperature: "temperature controlled"
Humidity: not reported
Air changes: not reported
Photoperiod: 12 hour light/dark

B. METHODS

Twenty-four hours prior to application, the dorsal area and trunk of 10 rabbits (5/sex) was clipped free of hair. On the day of dosing, 2000 mg/kg of the test substance was applied evenly over an area of approximately 10% of the body surface area and covered with a four layered surgical guaze patch (4 x 6 inches) and secured with non-irritating tape. The test article was weighed into individual doses and moistened slightly with distilled water to form a paste; the dose was based on the dry weight of the test substance. The paste was distributed evenly over the prepared skin site. Twenty-four hours later, the patches were removed and the application site washed off with distilled water. Immediately prior to dosing and on study days 7 and 14, the animals were weighed. The animals were observed for signs of clinical toxicity 1, 2, and 4 hours after application of the test substance and daily thereafter for the remainder of the 14 day study. The test sites were scored for dermal irritation at 24 hours after dosing and on days 7 and 14 using the numerical Draize scale (J.

Pharm. Exp. Ther. 82: 377-390, 1944). At the end of the study, the animals were euthanized (method not specified) and examined for gross pathology.

C. RESULTS

1. Mortality

No animals died during the study.

2. Clinical observations

Diarrhea and/or few feces were observed in all males and 2/5 females during days 6-14 of the observation period. A yellow nasal discharge was observed in one male on days 7-14. Two females exhibited erythema (one Draize Score 1-very slight, one Draize Score 2-well defined) at the test site on day 1; this erythema was absent on days 7 and 14.

3. Body weight

No significant changes in body weight were observed during the study.

4. Necropsy

One male rabbit exhibited yellow staining of the nasal area. No other effects were noted at necropsy.

5. LD_{50}

Based on the results of the study, the dermal LD_{50} of Rhizopon AA #3 (0.8% a.i.) for male and female New Zealand white rabbits is >2000 mg/kg.

D. Signed Quality Assurance and Good Laboratory Practice statements were present.

EPA Primary Reviewer: Sheryl K. Reilly, Ph.D. SKR, Date 6/22/95
EPA Secondary Reviewer: Roy D. Sjoblad, Ph.D. RDS, Date 6/27/95
Biopesticides and Pollution Prevention Division

DATA EVALUATION REPORT

STUDY TYPE: Primary Skin Irritation – Rabbit (152-14)

TOX. CHEM. NO.: 046701

DP BARCODE: D213970

MRID NO.: 435274-07

TEST MATERIAL: Rhizopon AA No. 3 (0.8% a.i.)

SYNONYMS: None known

STUDY NUMBER: MB 94-4093 C

SPONSOR: HORTUS USA CORP.

TESTING FACILITY: MB Research Laboratories, Inc., P.O. Box 178, Steinsburg and Wentz Roads, Spinnerstown, PA 18968

TITLE OF REPORT: Primary Dermal Irritation in Albino Rabbits

AUTHOR: Lori Kieffer

REPORT ISSUED: January 17, 1995 (Study completion date)

EXECUTIVE SUMMARY: Rhizopon AA No. 3 (0.8% a.i.), 0.5 g, was applied to the shaved dorsal trunk area of two male and four female New Zealand white rabbits. Four hours later, the patches were removed and the skin washed. The site of application was examined for erythema and edema 30 to 60 minutes and 24, 48, and 72 hours later. Erythema and edema were absent at all observations.

Based on the study results, Rhizopon AA No. 3 (0.8% a.i.) is **non-irritating** to the skin of male and female New Zealand white rabbits and is placed in **Toxicity Category IV**.

The study is classified as **Acceptable** and **meets** the requirements of Guideline Number 152-14 (81-5).

A. MATERIALS

1. Test material: Rhizopon AA No. 3 (0.8% a.i.)

   Description: gray powder
   Lot/Batch No.: unknown
   Composition: unknown
   Stability: unknown
   pH: unknown
   Solubility: water

2. Test animals

   Species: rabbit
   Strain: New Zealand white
   Age: approx 3-4 months
   Weight: pretest, 2.5-2.8 kg
   Source: Ace Animals, Boyertown, PA

3. Animal care

   Housing: individually in suspended cages
   Food: Purina Rabbit Chow (Diet No. 5321), assumed *ad libitum*
   Water: *ad libitum*
   Acclimation period: at least 7 days
   Temperature: "temperature controlled"
   Humidity: not reported
   Air changes: not reported
   Photoperiod: 12 hour light/dark

B. METHODS

Approximately 24 hours before application of the test material, the dorsal area of the trunk of 2 male and 4 female rabbits was clipped free of hair. A paste of 0.5 g of the test material in distilled water was evenly applied to a 6 cm^2 area of each animal, and the application site covered with a 1×1" gauze patch. The patch and the trunk of each animal were then wrapped with semi-occlusive tape. Four hours later, the patches were removed and the test sites wiped with water to remove residual test material. The sites were scored for erythema and edema 1, 24, 48, and 72 hours after patch removal according to the Draize method (J. Pharm. Exp. Ther. 82:377-390, 1944). The Primary Dermal Irritation (PDI) Index induced by the test material was determined by adding the average erythema and edema scores for each observation and dividing by the number of evaluation intervals. The animals were observed daily for signs of clinical toxicity and mortality during the 72-hour observation period.

EPA Reviewer: Sheryl K. Reilly, Ph.D. SKR, Date 6/28/95
EPA Reviewer: Roy D. Sjoblad, Ph.D. RDS, Date 6/27/95
Biopesticides and Pollution Prevention Division

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation - Rabbit (152-13)

TOX. CHEM. NO.: 046701

DP BARCODE: D213970

MRID NO.: 435274-08

TEST MATERIAL: Rhizopon AA No. 3 (0.8% a.i.)

SYNONYMS: None known

STUDY NUMBER: MB 94-4093 D

SPONSOR: HORTUS USA CORP.

TESTING FACILITY: MB Research Laboratories, Inc., P.O. Box 178, Steinsburg and Wentz Roads, Spinnerstown, PA 18968

TITLE OF REPORT: Primary Eye Irritation/Corrosion in Albino Rabbits

AUTHOR: Daniel R. Cerven

REPORT ISSUED: January 17, 1995 (Study completion date)

EXECUTIVE SUMMARY: In a primary eye irritation study, 0.1 ml equivalent Rhizopon AA No. 3 (0.8% a.i.) was instilled into one eye of 2 male and 4 female New Zealand white rabbits. The contralateral eye of each rabbit served as control. Each rabbit was examined 1, 24, 48, and 72 hours post-instillation for signs of ocular or clinial toxicity.

No corneal opacity was observed. Iritis was observed in 2/6 treated eyes at 1 hour and cleared by day 1. Conjunctival irritation was observed in 5/6 treated eyes and cleared by day 2. No clinical signs of toxicity were observed.

Based on the study, Rhizopon AA No. 3 (0.8% a.i.) is classified as **mildly irritating** to the eye of rabbits and is placed in **Toxicity Category III**. The study is classified as **Acceptable** and **meets** the requirements of Guideline Number 152-13 (81-4).

A. MATERIALS

1. Test material: Rhizopon AA No. 3 (0.8% a.i.)

   Description: gray powder
   Lot/Batch No. : unknown
   Composition: unknown
   Stability: unknown
   pH: unknown
   Solubility: water

2. Test animals

   Species: rabbit
   Strain: New Zealand white
   Age: approx. 4 months
   Weight: 2.3-2.8 kg
   Source: Ace Animals, Boyertown, PA

3. Animal care

   Housing: individually in suspended cages
   Food: Purina Rabbit Chow (Diet No. 5321), assumed *ad libitum*
   Water: *ad libitum*
   Acclimation period: at least 7 days
   Temperature: "temperature controlled"
   Humidity: not reported
   Air changes: not reported
   Photoperiod: not reported

B. METHODS

Prior to test material instillation, the eyes of animals in apparent good health (number not specified) were examined for gross anomalies using the Draize technique. Once found healthy, the test material, 0.1 ml equivalent (see study deficiencies, below) used as supplied, was instilled into the conjunctival sac of one eye of each rabbit and the upper and lower lids held together for approximately one second. The untreated contralateral eye served as control.

Ocular irritation was scored according to the method described by Draize et al. (J. Pharmacal, Exp. Ther. 83: 377-390, 1944) at 1, 24, 48, and 72 hours post-instillation. At the 24-hour observation interval, fluorescein dye was used to evaluate the extent of corneal damage. The primary eye irritation score for each rabbit was calculated by totaling corneal, iridal, and conjunctival scores. In addition to evaluating ocular damage, the animals were also observed daily for clinical signs of toxicity during the test period. Body weights were recorded pre-test. Body weights at the end of the study were not reported.

C. RESULTS

No corneal opacity was noted at any observation period. Iritis was observed in 2/6 treated eyes at 1 hour and cleared by day 1. Conjuctival irritation was observed in 5/6 treated eyes and cleared by day 2. Individual rabbit ocular irritation scores are found in Appendix A (MRID No. 435274-08, pp. 8-9). No clinical signs of toxicity were observed.

D. Signed Quality Assurance and Good Laboratory Practice statements were present.

E. STUDY DEFICIENCIES

The report states that 0.1 ml equivalent of the test article was instilled in each treated eye. However, no weight of the test compound is provided. This is a minor deficiency and is not thought to compromise the study.

APPENDIX A

MB RESEARCH LABS

PROT/PAGE : 236-04/8 of 10
PROJECT : MB 94-4093 D
TEST ARTICLE: Rhizopon AA #3 (0.8)

An.#/Sex:	ITEM	TISSUE	READING	HOUR 1	DAYS 1	2	3
E1292/M	A	Cornea	Opacity	0	0	0	0
	B		Area	0	0	0	0
		1. Total=(AxB)x5		0	0	0	0
	C	Iris		0	0	0	0
		2. Total = Cx5		0	0	0	0
	D	Conjunctiva	Redness	0	0	0	0
	E		Chemosis	0	0	0	0
	F		Discharge	0a	0	0	0
		3. Total=(D+E+F)x2		0	0	0	0
		Totals = 1+2+3		0	0	0	0
		SYSTEMIC OBSERVATIONS:		A	A	A	A
		SODIUM FLUORESCEIN :			0		
		PRETEST BODY WEIGHT : 2.7 kg					
E1305/M	A	Cornea	Opacity	0	0	0	0
	B		Area	0	0	0	0
		1. Total=(AxB)x5		0	0	0	0
	C	Iris		1	0	0	0
		2. Total = Cx5		5	0	0	0
	D	Conjunctiva	Redness	2	2	0	0
	E		Chemosis	2	0	0	0
	F		Discharge	2a	0	0	0
		3. Total=(D+E+F)x2		12	4	0	0
		Totals = 1+2+3		17	4	0	0
		SYSTEMIC OBSERVATIONS:		A	A	A	A
		SODIUM FLUORESCEIN :			0		
		PRETEST BODY WEIGHT : 2.5 kg					
E1268/F	A	Cornea	Opacity	0	0	0	0
	B		Area	0	0	0	0
		1. Total=(AxB)x5		0	0	0	0
	C	Iris		1	0	0	0
		2. Total = Cx5		5	0	0	0
	D	Conjunctiva	Redness	2	1	0	0
	E		Chemosis	2	0	0	0
	F		Discharge	2a	0	0	0
		3. Total=(D+E+F)x2		12	2	0	0
		Totals = 1+2+3		17	2	0	0
		SYSTEMIC OBSERVATIONS:		A	A	A	A
		SODIUM FLUORESCEIN :			0		
		PRETEST BODY WEIGHT : 2.3 kg					

A = normal a = small amount of residual test article remaining in conjunctiva

MB RESEARCH LABS

PROT/PAGE : 236-04/9 of 10
PROJECT : MB 94-4093 D
TEST ARTICLE: Rhizopon AA #3 (0.8)

An.#/Sex:	ITEM	TISSUE	READING	HOUR	DAYS 1	2	3
E1326/F	A	Cornea	Opacity	0	0	0	0
	B		Area	0	0	0	0
		1. Total=(AxB)x5		0	0	0	0
	C	Iris		0	0	0	0
		2. Total = Cx5		0	0	0	0
	D	Conjunctiva	Redness	2	0	0	0
	E		Chemosis	1	0	0	0
	F		Discharge	2a	0	0	0
		3. Total=(D+E+F)x2		10	0	0	0
		Totals = 1+2+3		10	0	0	0
		SYSTEMIC OBSERVATIONS:		A	A	A	A
		SODIUM FLUORESCEIN :			0		
		PRETEST BODY WEIGHT : 2.8 kg					
E1362/F	A	Cornea	Opacity	0	0	0	0
	B		Area	0	0	0	0
		1. Total=(AxB)x5		0	0	0	0
	C	Iris		0	0	0	0
		2. Total = Cx5		0	0	0	0
	D	Conjunctiva	Redness	2	0	0	0
	E		Chemosis	2	0	0	.
	F		Discharge	0a	0	0	0
		3. Total=(D+E+F)x2		8	0	0	0
		Totals = 1+2+3		8	0	0	0
		SYSTEMIC OBSERVATIONS:		A	A	A	A
		SODIUM FLUORESCEIN :			0		
		PRETEST BODY WEIGHT : 2.6 kg					
E1363/F	A	Cornea	Opacity	0	0	0	0
	B		Area	0	0	0	0
		1. Total=(AxB)x5		0	0	0	0
	C	Iris		0	0	0	0
		2. Total = Cx5		0	0	0	0
	D	Conjunctiva	Redness	2	0	0	0
	E		Chemosis	2	0	0	0
	F		Discharge	0	0	0	0
		3. Total=(D+E+F)x2		8	0	0	0
		Totals = 1+2+3		8	0	0	0
		SYSTEMIC OBSERVATIONS:		A	A	A	A
		SODIUM FLUORESCEIN :			0		
		PRETEST BODY WEIGHT : 2.4 kg					

A = normal a = small amount of residual test article remaining in conjunctiva

[RHIZOPON AA No. 3 (0.8)] Acute Inhalation Study (81-3)

EPA Reviewer: Sheryl K. Reilly, Ph.D. _____, Date 6/22/95
EPA Reviewer: Roy D. Sjoblad, Ph.D. _____, Date 6/27/95
Biopesticides and Pollution Prevention Division (7501W)

DATA EVALUATION REPORT

STUDY TYPE: *Acute Inhalation – Rat (152-12)*

TOX. CHEM. NO.: 046701

DP BARCODE: D213970

MRID NO.: 435274-09

TEST MATERIAL: Rhizopon AA No. 3 (0.8% a.i.)

SYNONYMS: None known

STUDY NUMBER: MB 94-4093 E

SPONSOR: HORTUS USA CORP.

TESTING FACILITY: MB Research Laboratories, Inc., P.O. Box 178, Steinsburg and Wentz Roads, Spinnerstown, PA 18968

TITLE OF REPORT: Inhalation Toxicity in Rats

AUTHOR: Daniel R. Cerven

REPORT ISSUED: January 17, 1995 (Study completion date)

EXECUTIVE SUMMARY: Five male and five female Wistar albino rats were exposed to a gravimetric atmospheric concentration of 2.4 mg/L Rhizopon AA No. 3 (0.8% a.i.) for four hours. Red staining of the nose/mouth area, closed eyes, coating of the fur with the test article and abnormal licking were observed during the exposure period. No other clinical signs of toxicity were observed; no effects on body weight were noted; and no animals died during the 14-day observation period. All animals were normal at necropsy. Based on the study results, the LC_{50} of Rhizopon AA No. 3 (0.8% a.i.) is **> 2.4 mg/L**, placing the test material in **Toxicity Category IV**.

The study is **Acceptable** and meets the requirement of Guideline Number 152-12 (81-3) for an acute inhalation toxicity study in rats.

A. MATERIALS

1. Test material: Rhizopon AA No. 3 (0.8% a.i.)

Description: gray powder
Lot/Batch No. : unknown
Composition: unknown
Stability: unknown
pH: unknown
Solubility: water

2. Test animals

Species: rat
Strain: Wistar
Age: approx. 2 months
Weight: pretest- 240-279 g (males), 209-240 g (females)
Source: Ace Animals, Boyertown, PA

3. Animal care

Housing: individually in suspended cages
Food: Purina Rat Chow No. 5012, *ad libitum* except during 4 hr exposure period
Water: *ad libitum* except during 4 hr exposure period
Acclimation period: at least 7 days
Temperature: "temperature controlled"
Humidity: not reported
Air changes: not reported
Photoperiod: 12 hour light/dark

B. METHODS

1. Exposure atmosphere generation

The test atmosphere was generated using a Venturi dust generator (Intox). Compressed air was supplied to the system at 4 psi.

2. Exposure chamber

A 57 liter glass exposure chamber was partitioned internally with wire screening into a total of 10 non-restraining cubicles. It was operated under a negative pressure. Chamber airflow was 50 liters per min (lpm), and yielded at least 10 to 15 air changes per hour so that adequate oxygen was supplied to the rats. The temperature and humidity of the exposure ch0amber were monitored every 30 minutes during the exposure period.

Gravimetric samples were withdrawn six times from the breathing zone of the animals during the exposure period to determine exposure concentration. Each sample was collected for 2 minutes by drawing chamber atmosphere under a 3 lpm vacuum onto a preweighed filter. After collection, the samples were again weighed to determine the mass collected. The mass collected was divided by the total volume of air sampled to determine chamber concentration.

Particle size distribution of the test atmosphere was determined twice during the exposure period using an 8-stage Andersen cascade impactor. (The time during exposure that the particle size distribution was collected was not reported). Samples of the test atmosphere were withdrawn from the breathing zone of the animals onto preweighed filter papers. The filter papers from each stage were weighed again after collection to determine the mass deposited. The aerodynamic mass median diameter and geometric standard deviation were determined graphically.

3. Experimental protocol

Wistar albino rats (5/sex) were placed into the chamber and exposed to the test atmosphere for 4 hours. Following exposure, the rats were washed with tap water. The animals were weighed immediately before exposure and on days 7 and 14 post-exposure. The animals were observed for signs of clinical toxicity at 1 hour intervals during exposure, and once daily thereafter for 14 days. On day 14 of the study, the rats were euthanized (method not specified) and examined for gross pathology.

C. RESULTS

1. Exposure conditions

The exposure conditions are summarized below.

Parameter	Results
Chamber Temperature	23.2-23.8°C
Chamber Humidity	47-50%
Chamber Airflow	50 lpm
Gravimetric Test Material Chamber Concentration	2.4 ± 0.39 mg/L
Mass Median Aerodynamic Diameter	
Average of Two Samples	3.4 ± 2.36 microns

2. Clinical observations

Red staining of the nose/mouth area, closed eyes, coating of the fur with the test article, and abnormal licking were reported during the exposure period only.

3. Body weight

No effects on body weight were reported.

4. Necropsy

Necropsy results were normal.

5. LC_{50}

None of the animals died during the 14-day observation period. Based on the study results, the male and female Wistar albino rat inhalation LC_{50} for Rhizopon AA No. 3 (0.8% a.i.) is > 2.4 mg/l.

D. Signed Quality Assurance and Good Laboratory Practice statements were presen

EPA Primary Reviewer: Sheryl K. Reilly, Ph.D. _____, Date 6/22/95
EPA Secondary Reviewer: Roy D. Sjoblad, Ph.D. _____, Date 6-22-95
Biopesticides and Pollution Prevention Division

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization, Buehler Method - Guinea Pig (152-15)

TOX. CHEM. NO.: 046701

DP BARCODE: D213970

MRID NO.: 435274-10

TEST MATERIAL: Rhizopon AA No. 3 (0.8% a.i.)

SYNONYMS: None known

STUDY NUMBER: MB 94-4093 F

SPONSOR: HORTUS USA CORP.

TESTING FACILITY: MB Research Laboratories, Inc., P.O. Box 178, Steinsburg and Wentz Roads, Spinnerstown, PA 18968

TITLE OF REPORT: Delayed Contact Dermal Sensitization Test - Buehler

AUTHOR: Theresa Newcomb

REPORT ISSUED: January 18, 1995 (Study completion date)

EXECUTIVE SUMMARY: Groups of 10 male Hartley guinea pigs were induced once per week (6 hours/treatment) for 3 weeks with either 0.4 g Rhizopon AA No. 3 (0.8% a.i.) moistened with 0.1 ml distilled water, or 0.2% 1-chloro-2,4-dinitrobenzene (DNCB) as a positive control. Two groups of 5 animals served as naive controls. Two weeks following the third induction, the animals were challenged with the moistened test material, or a 0.1% concentration of DNCB. Erythema was absent during induction and challenge with the test material. Positive control animals developed erythema both after induction and challenge with DNCB.

Based on the study results, Rhizopon AA No. 3 (0.8% a.i.) is **not a contact sensitizer** in Hartley guinea pigs.

The study is classified as **Acceptable** and **meets** the requirements of Guideline Number 152-15 (81-6).

A. MATERIALS

1. Test material: Rhizopon AA No. 3 (0.8% a.i.)

   Description: gray powder
   Lot/Batch No.: unknown
   Composition: unknown
   Stability: unknown
   pH: unknown
   Solubility: water

2. Positive control

   1-Chloro-2,4-dinitrobenzene (DNCB) (Sigma Chemical Co.)

3. Test animals

   Species: guinea pig
   Strain: Hartley
   Age: approx. 6 weeks
   Weight: males, 293-400 g pre-test
   Source: Ace Animals, Boyertown, PA

4. Animal care

   Housing: individually in suspended cages
   Food: Purina Guinea Pig Chow (Diet No. 5025), assumed *ad libitum*
   Water: assumed *ad libitum*
   Acclimation period: at least 5 days
   Temperature: "temperature controlled"
   Humidity: not reported
   Air changes: not reported
   Photoperiod: 12 hour light/dark cycle

B. METHODS

Preliminary irritation study to determine challenge dose

Since the test article as received (moistened with distilled water) did not produce irritation during induction, a screening study was not done to determine the irritancy potential of the test material for the challenge dose.

Induction - Two groups of 10 male animals were induced once a week for 3 weeks. Prior to each induction, the fur over the dorsal and flank area was clipped. The test material, 0.4 g moistened with distilled water, was applied to the left side of 10 animals using a 25 mm

Hilltop Chamber without the cotton pad. The positive control, 0.4 ml of 0.2% DNCB in 95% ethanol, was applied to the left side of the remaining 10 animals using a Hilltop Chamber with the cotton pad. The application chambers were secured in place and wrapped with adhesive tape for six hours. Due to severe reactions following induction # 2 in the positive controls, the dose site was moved to the right flank area for induction # 3. At the end of exposure, the chambers were removed and excess material washed from the site with distilled water. Skin reactions were recorded 24 and 48 hours after each induction using the following scoring system:

Score	Reaction
0	No erythema
0.5	Very faint erythema; usually non-confluent
1	Faint erythema; usually confluent
2	Moderate erythema
3	Strong erythema with or without edema.

Challenge - Fourteen days after the last induction, the animals were challenged at a naive site on the right flank in a manner similar to the inductions. Animals in the test material treatment group were challenged with 0.4 g Rhizopon AA No. 3 (0.8% a.i.) moistened with water, while those in the positive control group were challenged with 0.4 ml 0.1% DNCB in acetone. The challenge sites were evaluated for erythema 24, 48, and 72 hours later using the above scoring system.

In addition to the test material and positive control groups, 2 additional groups with five previously unexposed male guinea pigs each were added to serve as naive control groups. One group was challenged with the test material while the other was challenged with DNCB. The challenge sites on the naive controls were evaluated for erythema 24, 48, and 72 hours later using the above scoring system. A score of 2 or greater at challenge in 20% or more of the animals was considered indicative of a sensitizing response.

Body weights were recorded pre-test, 24 hours following the last induction, and 24 hours following the challenge application. Animals were observed once daily for mortality and toxicity

C. RESULTS

Induction - No erythema was observed during the induction phase of the study when animals were induced with 100% test material.

In the positive control, erythema, very faint to moderate following induction # 1, was moderate to severe following induction # 2. Due to the severity of the reaction following induction # 2, the dose site was moved for to induction # 3. Erythema was moderate to severe following induction # 3.

Challenge – No erythema was observed in any animals induced with the test material or in the naive controls challenged with test material. In the positive controls, erythema was faint to strong in the previously induced animals, and absent to moderate in the uninduced controls.

The test material was determined not to be a contact sensitizer to the skin of male guinea pigs, using the Buehler method.

D. Signed Quality Assurance and Good Laboratory Practice statements were present.

Note To File

Re. 63310-RO; 63310-EN; 63310-ER

Data matrix, product chemistry data, and confidential statement of formula are acceptable.

R. Kumar 4/7/95

Rita Kumar, RAL/BPPD

13544

R140448

Chemical: Indole-3-butyric acid

PC Code: 046701
HED File Code: 41500 BPPD Tox/Chem
Memo Date: 6/26/1995
File ID: DPD213970
Accession #: 000-00-9002

HED Records Reference Center
3/23/2007